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Cytochrome *bd* is a prokaryotic respiratory quinol oxidase identified in a number of pathogens, that is preferentially expressed under low O_2 tension or other "stress" conditions [1]. The enzyme couples the exergonic reduction of O_2 to 2 H_2O to proton motive force generation by transmembrane charge separation. Apart from its role in cell bioenergetics, evidence suggests that cytochrome *bd* accomplishes a number of additional functions of physiological relevance for the bacterial cell, being seemingly implicated also in microbial resistance to NO-stress (reviewed in [2]) and, in general, adaptation to the hostile conditions created by host immunity during the infection process.

Cytochrome *bd* from *Escherichia coli* contains three hemes, hemes b_{558} and b_{595} and heme *d*, where O_2 chemistry takes place through sequential formation of a few catalytic intermediates. Here, the isolated cytochrome *bd* was investigated by stopped-flow multi-wavelength absorption spectroscopy with the aim of measuring the occupancy of the catalytic intermediates at steady-state. We found that, under turnover conditions sustained by dithiothreitol-reduced ubiquinone and O_2, the ferryl and oxy-ferrous species are the mostly populated catalytic intermediates, with a minor fraction of the enzyme containing ferric heme *d* and possibly reduced heme b_{558} [3]. These new findings differ from those obtained with mammalian cytochrome *c* oxidase [4], where oxygen intermediates were not found to be populated at detectable levels under similar conditions. The results are discussed in the light of previously proposed models of the cytochrome *bd* catalytic cycle.

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14P7

Investigation of the proton pump and exit pathway in cytochrome ba_3 from *Thermus thermophilus*

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The heme-copper oxidases are redox driven proton pumps. These enzymes catalyze the reduction of O_2 to water and use the free energy of this reaction to generate a proton motive force. In this work we investigate the location of the proton loading site and exit pathway for pumped protons in the ba_3-type oxygen reductase from *Thermus thermophilus*. Previous studies suggested that one or both of the propionates of heme a_3 and residues in their vicinity may be important for these functions, and this was investigated by site directed mutagenesis. The data show that none of the amino acid residues near these propionates is essential for proton pumping or oxidase activity, including several residues previously postulated to be important: D372, H376 and $E126^{II}$ (in subunit II) and Y133. However, two mutants, D372I and H376N, exhibit high catalytic turnover and normal spectral features but do not pump protons or do so with noticeably diminished stoichiometry. It is concluded that the proton loading site might be either propionate-A of heme a_3 or a cluster of groups centered about a conserved water molecule that is hydrogen bonded to both of the heme a_3 propionates as well as to one of the histidine ligands to Cu_B.

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14P8

Rescue of complex IV biogenesis by the cytosol-synthesized subunit II (Cox2) precursor carrying the point mutation W56R

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Cytochrome *c* oxidase (complex IV or CcO) catalyzes oxygen reduction coupled to proton pumping. It is composed of 10–13 subunits and its biogenesis involves both mitochondrion- and nucleus-encoded polypeptides [1]. In yeast, as in the vast majority of eukaryotes, the three largest subunits of Cco are encoded in the mitochondrial genes COX1, COX2 and COX3. Deletion of the yeast COX2 gene results in loss of respiration (Δcox2 strain). Supekova et al. [2] were able to restore growth of a Δcox2 mutant in non-fermentable carbon sources after transformation with a vector expressing Cox2 with a mitochondrial targeting sequence and the point mutation W56R (Cox2W56R). In this work, the CcO carrying the allotopically-expressed Cox2W56R was studied. Isolated yeast mitochondria from the wild-type (WT) and the Δcox2 + Cox2W56R strains were solubilized and subjected to Blue Native electrophoresis. In-gel activity of CcO and spectroscopic quantitation of cytochromes revealed that only 40% of complex IV is present in the complemented strain as compared to the wild-type strain. CcOs from the WT and the rescued mutant exhibited similar subunit composition, although activity was 20–25% lower in the enzyme containing Cox2W56R than in the one containing native Cox2 (Cox2WT). Tandem mass spectrometry confirmed that W56 was substituted by R56 in Cox2W56R. In addition, as judged by Edman degradation, Cox2W56R exhibited the same N-terminus than Cox2WT, indicating that both the MTS of Oxa1 and the leader sequence of 15 residues were removed from Cox2W56R during maturation. Thus, Cox2W56R is identical to Cox2WT except for the point mutation W56R. Mitochondrial Cox1 synthesis is strongly reduced in Δcox2 mutants, but the